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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/540,814	01/09/2006	Kenji Miyazaki	18962	6341

23389 7590 11/17/2009
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EXAMINER	
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ART UNIT	PAPER NUMBER
1797	

MAIL DATE	DELIVERY MODE
11/17/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/540,814	MIYAZAKI ET AL.	
	Examiner	**Art Unit**	
	ROBERT XU	1797	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 October 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-18* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

1. The amendment and RCE filed on 10/14/2009 has been entered and fully considered. Claims 1-18 are pending, of which Claims 1, 5-7 and 18 are amended.

Response to Amendment

2. In response to amendment, the examiner maintains rejection over the prior art established in the previous Office action.

Claim Rejections - 35 USC § 103

3. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

4. **Claims 1, 2, 4-6 and 18** are rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita et al. (Electrophoresis, 1998) (Tsugita) in view of Covey et al. (US Patent 5,952,653) (Convey) and Xu et al. (Analytical Biochemistry, 1997) (Xu).

In regard to Claims 1 and 18, Tsugita teaches a method of analyzing C-terminal sequence of a peptide by means of mass spectrometry.

Tsugita teaches pretreatment step using acetic anhydride react with the dried peptide to acetylate the N-terminus and form an oxazolone at the C-terminal carboxyl group (see page 930, right col. 3rd paragraph). While Tsugita does not specifically teach acetylation of the side chain of lysine residue, acetylation of the side chain of lysine residue is the inherent result of reacting acetic anhydride with peptide that has lysine residue.

Tsugita teaches a step of allowing acetic anhydride to act on the dry peptide in the presence of pentafluoropropionic methyl ester (PFPMe) to successively release the C-terminal amino acid by the cleavage of the 5-oxazolone ring (see page 930, right col. 3rd paragraph). PFPMe is similar to pentafluoropropionic acid (PFPA) in structure and reaction. Tusgita also teaches using PFPA in the cleavage of predetermined position of the peptide (see page 932, left col. 4th paragraph). It would have been obvious to ordinary skill in the art to substitute PFPMe with PFPA in the step of releasing the C-

terminal amino acids in Tsugita method, because these compounds have similar structures and similar effects.

Tsugita teaches hydrolysis treatment step by allowing water molecules to act on the C-terminal-deleted peptides in the presence of dimethylamino ethanol (DMAE) (see page 930, right col. 3rd paragraph).

Tsugita teaches measuring the decreases in molecular weight associated with the successive release of the C-terminal amino acids by mass spectra (see Table 3).

Tsugita does not specifically teach allowing trypsin to act on the sample to cleave peptide for mass spectrometer analysis. Tsugita teaches that highly specific proteases have been used for specific fragmentation in the peptide-mass fingerprinting technique (see page 931, left col. 2nd paragraph). Trypsin is one of highly specific proteases commonly used. Covey teaches using trypsin to cleave peptide for mass spectrometry analysis (see abstract). Covey further teaches that the tryptic fragments ions are predominantly doubly charge cationic species, because arginine and lysine are both very basic and each picks up a positive charged proton in solution (see col. 2. lines 63-67; Col. 3, lines 39-42), and the C-terminal tryptic fragment not containing an arginine or a lysine is single charged (see col. 5, lines 58-60). At the time of the invention, it would have been obvious to one of ordinary skill in the art to use trypsin to cleave the peptide before mass spectrometry analysis, as taught by Covey, in the method of Tsugita, because smaller peptides are easier to analyze by mass spectrometry.

Tsugita does not teach the protocol of analyzing mass spectra by comparing the peaks of cationic species with the peaks of the anionic species as described in the steps 1-9 in the instant claim. As has been discussed above, Covey teaches that tryptic fragments ions are predominantly doubly charge cationic species because arginine and lysine are both very basic and each picks up a positive charged proton in solution (see col. 2. lines 63-67; Col. 3, lines 39-42), and the C-terminal tryptic fragment not containing an arginine or a lysine is single charged (see col. 5, lines 58-60). The method of analyzing mass spectra according to the expected charges of the species is well known in the art.

Xu teaches that fragmentation patterns in positive mode and negative mode are complementary for the elucidation of the peptide chain sequence (see page 10-13, *MALDI-PSD Analysis*). Xu further teaches correlating peaks that only different in a chemical group, e.g. OH (mass of 18) or N-acetyl glucosamine (MurNAC mass of 203) because of the reaction (see page 10, right col.). Xu's teaching is similar to the criteria 5a-1, 5a-2, 5a-3, 5b-1, 5b-2, 5b-3 of the instant claim.

Applicant is advised that the rationale to support a conclusion that the claim would have been obvious is that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination yielded nothing more than predictable results to one of ordinary skill in the art. (see KSR, 550 U.S. at ___, 82 USPQ2d at 1395) (see MPEP 2143). In that regard, one of ordinary skill in the art could have utilized the charge difference between normal tryptic fragments and C-terminal tryptic fragment as taught by Covey, and correlated the fragmentation patterns in positive mode and negative mode in relative intensity and further correlated with the special group difference due to chemical reaction, as taught by Xu to elucidate the peptide chain sequence by calculating the mass difference between the C-terminal successive deleted peptides as taught by Tsugita, with the predictable result.

In regard to Claim 2, it is well known in the art that spiked noise in mass spectra usually has narrower full-width of half maximum than normal peak of signal. Therefore, removing peaks of spiked noise based on full-width of half maximum would have been obvious to ordinary skill in the art. The peak smoothing and smoothing algorithms are also well known in the art. Therefore, peak smoothing would have been obvious to ordinary skill in the art.

In regard to Claim 4, the normal tryptic fragments carry two positive charges as taught by Covey. Under the same reason, when C-terminal fragment has arginine at the C-terminal (CFAC), it will also carry two positive charges. However, just like other C-terminal fragments, CFAC will also have an adjacent fragment peak that has molecular weight difference from CFAC equals formula weight of natural chain amino acid or acylated amino acid as taught by Tsugita. It would have been obvious to ordinary skill

in the art to use the criteria of adjacent peaks to judge if the strong cationic peak is CFAC based on teaching of Tsugita and Convey.

In regard to Claim 5, Tsugita teaches measuring the decrease in molecular weight associated with successive release of the C-terminal amino acids (see Table 3). Tsugita teaches using MALDI-TOF-MS for the measurement (see page 931, left col. 2nd paragraph). Xu teaches considering fragmentation patterns in both cationic species (positive mode) and anionic species (negative modes) (see abstract). At the time of the invention, it would have been obvious for ordinary skill in the art to consider both cationic species and anionic species as taught by Xu in Tsugita's method to obtain the peptide sequence, because Xu specifically teaches that the fragmentation in positive mode and negative mode are complementary for elucidation of the peptide sequence.

In regard to Claim 6, Tsugita teaches a process for releasing the C-terminal amino acids successively. Tsugita teaches pretreatment step using acetic anhydride and acetic acid vapor react with the dried peptide at 60ºC to acetylate the N-terminus and form an oxazolone at the C-terminal carboxyl group (see page 930, right col. 3rd paragraph). Tsugita does not specifically teach acetylation of the side chain of lysine residue. Acetylation of the side chain of lysine residue is the inherent result of reacting acetic anhydride with peptide that has lysine residue.

Tsugita teaches a step of allowing acetic anhydride vapor to act on the dry peptide in the presence of pentafluoropropionic methyl ester (PFPMe) vapor to successively release the C-terminal amino acid by the cleavage of the 5-oxazolone ring at 5ºC (see page 930, right col. 3rd paragraph). PFPMe is similar to pentafluoropropionic acid (PFPA) in structure and reaction. Tsugita also teaches using PFPA in the cleavage of predetermined position of the peptide (see page 932, left col. 4th paragraph). It would have been obvious to ordinary skill in the art to substitute PFPMe with PFPA in the step of releasing the C-terminal amino acids. Tsugita allows the reaction at 5ºC. The court has held that [W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation (*In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)). In that regard, although

5ºC is outside the range of 15-60ºC in the instant claim, it would have been obvious for ordinary skill in the art to optimize the reaction temperature by routine experimentation.

Tsugita does not literally teach removing the remaining alkanoic acid anhydride and perfluoroalkanoic acid in a dry state at the end of C-terminal cleaving reaction. However, this removing step at the end of the reaction is inherent part of the reaction step, because when the reaction is complete, the remaining reagents should be removed. Tsugita teaches hydrolysis treatment step by allowing water molecules to act on the C-terminal-deleted peptides in the presence of dimethylamino ethanol (DMAE). (see page 930, right col. 3rd paragraph). Again, the removing remaining basic nitrogen-containing organic is inherent part of the reaction step.

Tsugita does not specifically teach allowing trypsin to act on the sample to cleave peptide for mass spectrometer analysis. Tsugita teaches that highly specific proteases have been used for specific fragmentation in the peptide-mass fingerprinting technique (see page 931, left col. 2nd paragraph). Trypsin is one of highly specific proteases commonly used. Covey teaches using trypsin to cleave peptide for mass spectrometry analysis (see abstract). Covey further teaches that the tryptic fragments ions are predominantly doubly charge cationic species because arginine and lysine are both very basic and each picks up a positive charged proton in solution (see col. 2. lines 63-67; Col. 3, lines 39-42), and the C-terminal tryptic fragment not contain an arginine or a lysine is signally charge (see col. 5, lines 58-60). Since the side chain of lysine is protected by acetylation treatment as discussed above, only arginine site will be cut by trypsin. At the time of the invention, it would have been obvious to one of ordinary skill in the art to use trypsin to cleave the peptide before mass spectrometry analysis as taught by Covey in the method of Tsugita, because smaller peptides are easier to analyze by mass spectrometry.

Removing trypsin at the end of the reaction is inherent part of the reaction. Desalting treatment is commonly used for changing buffer or removing buffer solution component. It would have been obvious to ordinary skill in he art to use desalting treatment to remove the trypsin from the buffer solution to stop the reaction. Drying sample is a required step before performing MALDI-TOF-MS.

Tsugita teaches using MALDI-TOF-MS for measuring the molecular weight of the peptide fragments (see page 931, left col. 2nd paragraph). Tsugita does not teach analyzing mass spectra by comparing the peaks of cationic species with the peaks of the anionic species. As has been discussed above, Covey teaches that tryptic fragments ions are predominantly doubly charge cationic species because arginine and lysine are both very basic and each picks up a positive charged proton in solution (see col. 2. lines 63-67; Col. 3, lines 39-42), and the C-terminal tryptic fragment not containing an arginine or a lysine is signally charge (see col. 5, lines 58-60). The method of analyzing mass spectra according to the expected charges of the species is well known in the art. Xu teaches that fragmentation patterns in positive mode and negative mode are complementary for the elucidation of the peptide chain sequence (see abstract). It would have been obvious to one of ordinary skill in the art to utilize the charge difference between normal tryptic fragments and C-terminal tryptic fragment as taught by Covey and correlate the fragmentation patterns in positive mode and negative mode as taught by Xu to elucidate the peptide chain sequence by means of MALDI-TOF-MS as taught by Tsugita.

5. **Claim 3 is** rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita in view of Covey and Xu, as applied to claims 1-2 and 4-6 above, and further in view of Harris et al. (Rapid Communications in mass spectrometry, 2002) (Harris).

In regard to Claim 3, Tsugita in view of Convey and Xu do not teach using the fragments of trypsin autolysis as the internal standard to calibrate mass spectra. The molecular weights and charges of peptide fragments derived from the trypsin autolysis are well known in the art. Harris teaches using trypsin autolysis fragments as mass calibrants in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (see abstract). Therefore, it would have been obvious to ordinary skill in the art to modify Tsugita-Convey-Xu's method by calibrating mass spectra using the peptide fragments of trypsin autolysis, as taught by Harris.

6. **Claims 7-17** are rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita in view of Covey and Xu as applied to 1,2 and 4-6 above, and further in view of Vogt et al. (Polymer Bulletin, 1996) (Vogt)

In regard to Claim 7, Tsugita teaches separating target peptide by electrophoresis before process for C-terminal analysis (see page 929, right col. 2nd paragraph). Tsugita in view of Convey and Xu do not teach releasing the C-terminal amino acids successively while the peptide is bound on a gel carrier. Tsugita teaches that the pretreatment and cleavage sub-step of the procedure needs to be carried out in the absence of water (page 931, right col. 2nd paragraph, last 2 lines; page 930, 3rd paragraph). Therefore, the target protein has to be extracted from the gel and then dried to remove water or electroblotted to an Immobilon-CD membrane. The consequence of extracting peptide from a gel after separation is that some peptide will be lost during the process (it is true to any peptide or protein processing). Therefore, sequencing the peptide while it is bound to a gel would avoid the loss of peptide during extraction.

Tsugita does not teach a step of using polar aprotic solvent to remove water. However, using polar aprotic solvent to remove water is known in the art.

Tsugita teaches applying N-acylation protection by alkanoic acid anhydride at 60ºC (see page 930, right col. 3rd paragraph). Tsugita does not teach using dipolar-aprotic solvent to swollen the gel so that the pretreatment and C-terminal cleavage reaction could be carried out on the protein bound to the original gel right after electrophoresis. Vogt teaches a new non-aqueous swelling system; specifically he teaches that carboxymethyl cellulose (CMC) gel treated with a dipolar aprotic solvent like *N,N*-dimethylacetamide with *p*-toluenesulfonic acid yields a high reactive gel-suspension of the polymer (see abstract). This dipolar aprotic solvent can remove water from the swollen gel in one step (see page 550, 3rd paragraph), thus allowing a direct esterification of the hydroxyl group of CMC (see abstract). At the time of the invention, it would have been obvious to one of ordinary skill in the art to use polar aprotic solvent to remove water and use dipolar aprotic solvent to remove water from the gel carrier bound with the target protein, as taught by Vogt with reasonable expectation that this would allow Tsugita-Convey-Xu's procedure to be carried out on the target protein kept on the gel carrier. Sequencing the peptide while it is bound to a gel would avoid the loss

of peptide during extraction. Also because Vogt specifically teaches that dipolar aprotic solvent can remove water from gel while keep the gel swollen.

Tsugita teaches a step of allowing acetic anhydride to act on the dry peptide in the presence of pentafluoropropionic methyl ester (PFPMe) to successively release the C-terminal amino acid by the cleavage of the 5-oxazolone ring (see page 930, right col. 3rd paragraph). PFPMe is similar to pentafluoropropionic acid (PFPA) in structure and reaction. Tusgita also teaches using PFPA in the cleavage of predetermined position of the peptide (see page 932, left col. 4th paragraph). It would have been obvious to ordinary skill in the art to substitute PFPMe with PFPA in the step of releasing the C-terminal amino acids in Tsugita method, because these compounds have similar structures and similar effects.

Tsugita does not teach using polar aprotic solvent to remove dipolar aprotic solvent. Polar aprotic solvent and dipolar aprotic solvent are both aprotic solvents and very similar. It is well know that they can be used to remove each other.

Tsugita teaches hydrolysis treatment with an aqueous solution of tertiary amine compound (DMAE) after successive release of C-terminal amino acids (see page 930, right col. 3rd paragraph).

Tsugita does not specifically teach allowing trypsin to act on the sample to cleave peptide for mass spectrometer analysis. Tsugita teaches that highly specific proteases have been used for specific fragmentation in the peptide-mass fingerprinting technique (see page 931, left col. 2nd paragraph). Trypsin is one of highly specific proteases commonly used. Covey teaches using trypsin to cleave peptide for mass spectrometry analysis (see abstract). Covey further teaches that the tryptic fragments ions are predominantly doubly charge cationic species because arginine and lysine are both very basic and each picks up a positive charged proton in solution (see col. 2. lines 63-67; Col. 3, lines 39-42), and the C-terminal tryptic fragment not contain an arginine or a lysine is signally charge (see col. 5, lines 58-60). Since the side chain of lysine is protected by acetylation treatment, only arginine site will be cut by trypsin. At the time of the invention, it would have been obvious to one of ordinary skill in the art to use trypsin to cleave the peptide before mass spectrometry analysis as taught by Covey in the

method of Tsugita, because smaller peptides are easier to analyze by mass spectrometry.

Desalting treatment is commonly used for changing buffer or removing buffer solution component. It would have been obvious to ordinary skill in he art to use desalting treatment to remove the trypsin from the buffer solution to stop the reaction. Drying sample is a required step before performing MALDI-TOF-MS.

Tsugita teaches using MALDI-TOF-MS for measuring the molecular weight of the peptide fragments (see page 931, left col. 2nd paragraph). Tsugita does not teach analyzing mass spectra by comparing the peaks of cationic species with the peaks of the anionic species. As has been discussed above, Covey teaches that tryptic fragments ions are predominantly doubly charge cationic species because arginine and lysine are both very basic and each picks up a positive charged proton in solution (see col. 2. lines 63-67; Col. 3, lines 39-42), and the C-terminal tryptic fragment not containing an arginine or a lysine is signally charge (see col. 5, lines 58-60). The method of analyzing mass spectra according to the expected charges of the species is well known in the art. Xu teaches that fragmentation patterns in positive mode and negative mode are complementary for the elucidation of the peptide chain sequence (see abstract). It would have been obvious to one of ordinary skill in the art to utilize the charge difference between normal tryptic fragments and C-terminal tryptic fragment as taught by Covey and correlate the fragmentation patterns in positive mode and negative mode as taught by Xu to elucidate the peptide chain sequence by means of MALDI-TOF-MS as taught by Tsugita.

In regard to Claims 8 and 9, Tsugita teaches that 20% acetic anhydride is used in the first step of the procedure for applying N-acetylation protection to the N-terminal of the protein and for forming oxazolone at C-terminal of the protein and 5% PFPMe is used in the second step to react with oxazolone (page 930, right col. 3rd paragraph). Tsugita does not specifically teach maintaining acetic anhydride in the second step. However, since the function of acetic anhydride is to form oxazolone at C-terminal for perfluoroalkanoic acid to act on in the second step, it would have be obvious to ordinary skill in the art to recognized that maintaining the concentration of acetic anhydride in the

second step may benefit the reaction. Therefore, modified method of Tsugita teaches using symmetric anhydride of linear –chain alkanoic acid having 2 carbons (acetic anhydride) for the formation of 5-oxazolone and subsequently release of C-terminal amino acids in association with cleavage of the 5-oxazolone ring (see page 930, right col. 3rd paragraph).

In regard to Claim 10, Tsugita teaches using acetic anhydride as the alkanoic acid anhydride (see page 930, right col. 3rd paragraph).

In regard to Claim 11, the pKa of PFPA is in a range of 0.3 to 2.5.

In regard to Claim 12, PFPA has 3 carbon atoms.

In regard to Claim 13, modified method of Tsugita teaches that the ratio of PFPA (5%) to acetic anhydride (20%) would be 1:4 (see page 930, right col. 3rd paragraph). In the instant Claim, the lower limit of the ratio is 20:100 or 1:5. Applicant is advised that generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). Therefore, it would have been obvious to one of ordinary skill in the art to discover the optimum ratio of perfluoroalkanoic acid to alkanoic acid anhydride by routine experimentation.

In regard to Claims 14-16, Tsugita teaches that acetic anhydride is used in the pretreatment step of applying N-acylation protection (see page 930, right col. 3rd paragraph).

In regard to Claim 17, Tsugita teaches using acetic anhydride as the alkanoic acid anhydride in the pretreatment step of applying N-acylation protection. Modified method of Tsugita teaches using acetic anhydride in combination with PFPA for the formation of 5-oxazolone and subsequent release of C-terminal amino acids in association with the cleavage of the 5-oxazolone ring (see page 930, right col. 3rd paragraph).

Response to Arguments

7. Applicant's arguments filed 10/14/2009 have been fully considered but they are not persuasive.

Applicant argues that "Tsugita does not teach measuring the mass/charge of the cationic species of $(M+H)^+$ as well as the mass/charge measurement for the anionic species of $(M-H)^{-"}$. Tsugita might not teach mass/charge measurement for the anionic species of $(M-H)^-$, Tsugita teaches measuring the mass/charge of the cationic species of $(M+H)^+$ (see page 932, left col. 1st paragraph).

The applicant argues that "Covey fails to provide any suggestion as to whether or not such a double charge rule will be also observed for MALDI-TOF-MS or FRB-MS". Although Covey does not specifically teach MALDI-TOF-MS, ordinary skill in the art would know that the double charge rule taught by Covey is true for all ion evaporation mass spectrometry of Tryptic fragments. MALDI-TOF-MS or FRB-MS is not an exception.

Applicant argues that "Xu is limited to the use of $(M-H)^-$ values for molecular weight and nothing more. Thus, Xu fails to suggest that the spectrum observed in the positive mode would be suitably used in combination with the spectrum observed in the negative mode to identify the mass of each of the fragments contained in the analysis solution". Xu teaches the positive mode MALDI spectrum in Figure 3 and 4 and negative mode of MALDI spectrum in Figure 5 and 6. Thus, Xu demonstrates that sensitive detection can be achieved for both positive and negative mode for MALDI-MS. Xu teaches that extension of MALDI-TOF-MS allows structural information to be obtained in addition to molecular weight data by means of so called post source decay (PSD) analysis (see page 8, left col. 2nd paragraph). Xu shows that fragmentation patterns in positive mode and negative mode PSD were complementary for the elucidation of the peptide chain sequence (see page 10-13, *MALDI-PSD Analysis*).

Applicant argues that "Harris teaches a method of using the $(M+H)^+$ ions from the trypsin autolysis fragments as mass calibrants in the positive-ion mode MALDI-TOF based analysis. Harris fails to provide any experimental evidence suggesting that the $(M-H)^-$ ions from the trypsin autolysis fragments will be successfully used as mass

calibrants in the negative-ion mode MALDI-TOF based analysis." The mass (M) of a trypsin autolysis fragment should be the same no matter it is measured in a positive mode $(M+H)^+$ (M+1) or a negative mode $(M-H)^-$ (M-1) of MALDI-TOF. The possibility of success of using trypsin autolysis fragments as mass calibrants in the negative-ion mode MALDI-TOF based analysis would have been reasonably expected by one of ordinary skill in the art.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROBERT XU whose telephone number is (571)270-5560. The examiner can normally be reached on Mon-Thur 7:30am-5:00pm, Fri 7:30am-4:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Vickie Kim can be reached on (571)272-0579. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

11/14/2009

/Yelena G. Gakh/
Primary Examiner, Art Unit 1797

RX